

## **Supplementary method and figures**

### **TRPV1 Channel in Human Eosinophils: Functional Expression and Inflammatory Modulation**

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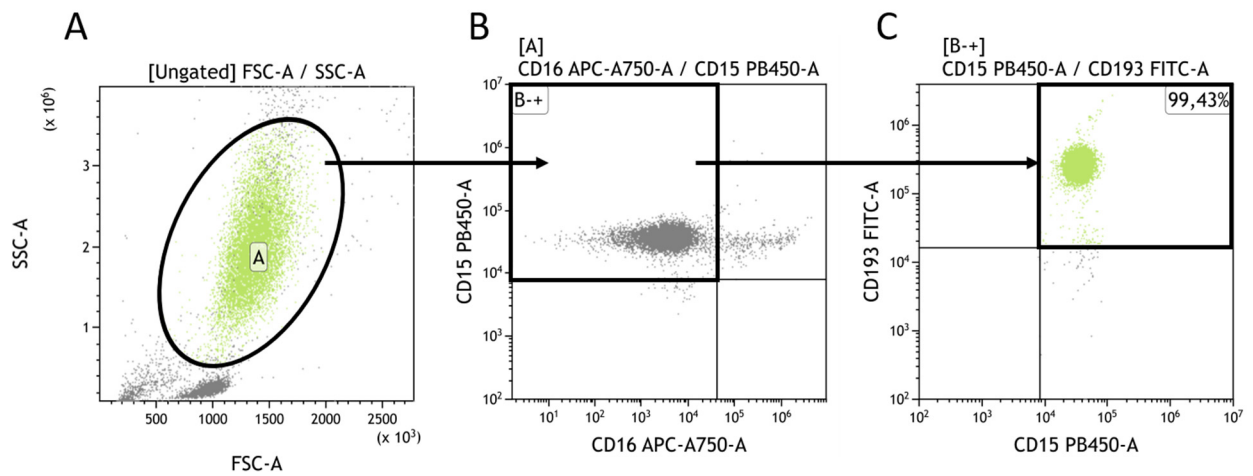
## **Supplementary Method**

### **Stimulation of eosinophils with IL-13 and capsaicin**

Purified eosinophils were resuspended in RPMI medium (containing 10 % and 1 % PenStrep). Eosinophils were stimulated for 24 h at 37 °C and 5 % CO<sub>2</sub> with 0.1, 1, 10, or 100 µM capsaicin. Further, we stimulated eosinophils for 4 h at 37 °C and 5 % CO<sub>2</sub> with IL-13 (50 ng/ml). TRPV1 surface expression was determined using a TRPV1-PE (Biozol, Eching, Germany) antibody and flow cytometry analysis.

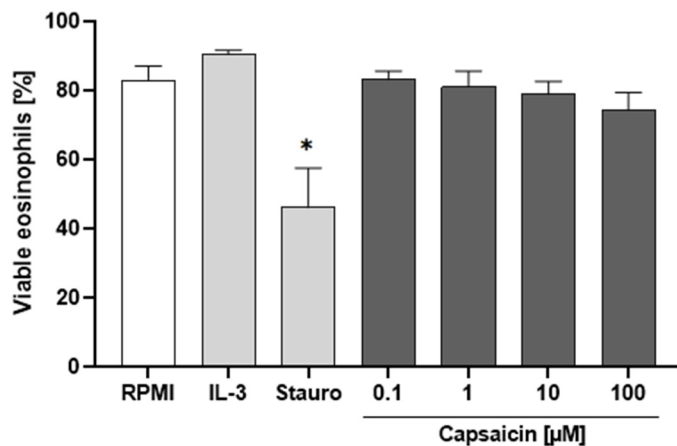
## Figure Supplement

S1



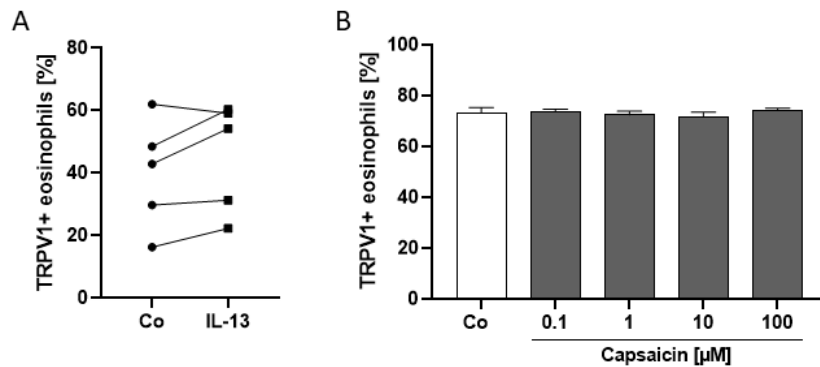
**Figure S1. Gating strategy of eosinophil selection for surface content analyses.** (A) Granulocyte population was selected for determining (B) CD15<sup>+</sup>, CD16<sup>-</sup>, and (C) CD193<sup>+</sup> eosinophils. Representative dot plots of one healthy blood donor.

S2



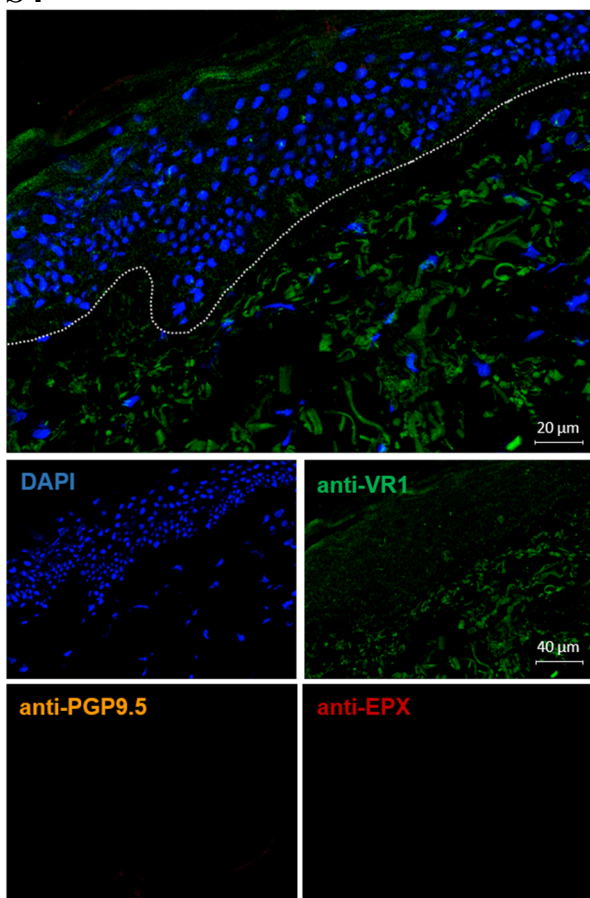
**Figure S2. Detection of apoptosis 4 h after TRPV1 activation.** Percentage of viable eosinophils from healthy donors after incubation with capsaicin (0.1, 1, 10, 100  $\mu$ M), IL-3 (10 ng/ml) as an anti-apoptotic, staurosporine (1  $\mu$ M) as a pro-apoptotic, or RPMI medium as the negative control (Co) for 4 h at 37  $^{\circ}$ C and 5 % CO<sub>2</sub>. Eosinophils were stained with Annexin V and propidium iodide and fluorescence was measured through flow cytometry (n=5; \* = p < 0.05;  $\pm$ SEM).

S3



**Figure S3. IL-13 and TRPV1 activation does not change TRPV1 surface content on eosinophils.** (A) Percentage of TRPV1+ eosinophils of five patients after stimulation with IL-13 (50 ng/ml) or RPMI medium as the negative control (Co) for 4 h at 37 °C and 5 % CO<sub>2</sub>. TRPV1 expression was assessed through flow cytometry (n=5). (B) Percentage of TRPV1+ eosinophils after stimulation with capsaicin (0.1, 1, 10, 100  $\mu$ M) or RPMI medium as the negative control (Co) for 24 h at 37 °C and 5 % CO<sub>2</sub> (n=3;  $\pm$ SEM).

S4



**Figure S4. Immunofluorescence staining in non-inflamed skin.** Skin sections obtained from healthy subjects were fixed with methanol and stained with anti-TRPV1 (green), anti-EPX (red) as the eosinophil marker, and anti-PGP9.5 (orange) as the neuronal marker. Cell nuclei were labeled with DAPI (blue). Skin section was analyzed at 40x magnification through fluorescence microscopy. Representative staining out of n=3 healthy donors.

## References

1. Limberg MM, Wiebe D, Gray N, Weihrauch T, Bräuer AU et al. (2024) Functional expression of TRPV1 in human peripheral blood basophils and its regulation in atopic dermatitis. *Allergy* 79 (1): 225-228.